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Metabolism of Methionine by Certain Tissues of the Intact Chick.

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METABOLISM OF METHIONINE BY CERTAIN TISSUES OF THE INTACT CHICK

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To his wife, Mary Nell, he wishes to say simply--thank you.

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ABSTRACT

These experiments were initiated to study the metabolism of chicks as influenced by various levels of methionine ranging from below to well above the established requirement.

The criteria employed were weight gain, feed consumption, and the concentration of radioactivity localized within various organs.

The metabolism of $C^{14}H_3$-methionine and methionine-2-C^{14} was traced by sacrificing the chick and determining the radioactivity contributed by each source of radioactive carbon in blood, muscle tissue, kidney, and the liver.

The liver was also subfractionated by differential centrifugation into nuclear, mitochondrial, microsomal, and soluble protein fractions, and the distribution of radioactivity within these subfractions was also determined.

Composite samples were made of the soluble protein fractions and these were subjected to amino acid analysis by ion exchange chromatography. These amino acid samples were converted into $C^{14}O_2$ and the amount of radioactivity determined in a scintillation counter.

From these experiments it was found that optimum weight gain and feed conversion were observed at 0.84 percent dietary methionine (0.09 percent cysteine), and that feed

consumption was depressed by methionine levels above or below 0.84 percent.

It was further noted that the efficiency of utilization of methionine was decreased as the amount in the diet increased and that above optimum dietary levels of methionine tended to depress growth.

Recovery of radioactive carbon from the various tissues and organs indicated that rapid transmethylation was occurring at all dietary levels but a true preferential utilization of methionine for one-carbon metabolism or protein synthesis at a particular dietary level was not demonstrated.

INTRODUCTION

From the time of its isolation in 1922, methionine has been of considerable interest as an essential component in the formulation of poultry rations.

In many instances methionine supplementation has been reported to effect an increase in weight gain and feed utilization. However, it has also been found that levels of this amino acid above the established requirement may result in decreased weight gain and feed efficiency.

Methionine has been observed to function as a free amino acid and as a constituent of protein. Also, it has been known for many years that the labile methyl group of methionine is intimately involved with single carbon metabolism.

In this study isotopically labeled methionine was employed to determine whether there was a preferential utilization of methionine for one or the other of these functions.

REVIEW OF LITERATURE

An improvement in growth and feed efficiency has been obtained by supplementing poultry rations with certain amino acids. Methionine has been of particular interest in this respect.

Klose and Almquist (1941) and Featherson and Stephenson (1960) showed methionine to be an indispensable amino acid for normal growth and development of the chick. The latter investigators also reported that methionine was interrelated with various other factors.

Rosenberg et al. (1955) reported an addition of 0.05 percent DL-methionine to a corn-soy type ration supplemented with three to six percent fat resulted in improved growth and feed efficiency. The response when both ingredients were present was greater than when either fat or methionine was added separately.

Rosenberg and Baldini (1957), using isocaloric diets of different protein levels, indicated that the methionine requirement was related to the energy content of the diet. If sufficient energy were available from non-protein sources to allow complete utilization of the protein for tissue repair and synthesis, the methionine requirement (expressed as a percent of the diet) increased as the protein level

increased. These workers also presented data which showed the performance of chicks fed a 20 percent protein diet improved with the addition of methionine to rations containing either 903 Calories of PE per pound or 1003 Calories of PE per pound. Maximum performance was reached for the lower calorie ration with 0.433 percent total methionine and at 0.508 percent total methionine with the higher calorie ration. This work was in agreement with that of Almquist (1949) who demonstrated that the methionine requirement was approximately 0.75 percent when a 30 percent protein diet was fed. This was the proportional level corresponding to the established methionine requirement (0.45 percent) when a 20 percent protein diet was fed.

Waibel (1959), was not able to obtain faster growth with Broad-Breasted Bronze poults by increasing the protein level from 28 to 32 percent, or by adding 10 percent dietary fat in practical type diets. He found that methionine was the first limiting amino acid at a protein level of 30 percent in a corn-soy diet and supplementation with this amino acid resulted in consistent growth responses.

Broad-Breasted Bronze poults were fed high and low protein rations up to 16 weeks of age. The effects of methionine supplementation were slightly greater with high energy diets. Balloun (1962) concluded that methionine tended to exert its effect during the first six weeks of the growing period.

A methionine deficient semi-purified diet was

evaluated by Carew and Hill (1961). They observed a significant increase in feed consumption and fat deposition as compared to the chicks which received the methionine adequate diet. A significant difference in growth was not noted.

Quillin et al. (1961) suggested that methionine produced a significant growth response in chicks only with a low choline ration. Furthermore, the improvement in growth with the addition of choline approached statistical significance only with rations marginal in methionine. The addition of both of these materials was not significantly better than the supplementation with either choline or methionine at the highest levels used.

White and Beach (1937) demonstrated that homocystine could stimulate growth of rats fed a methionine deficient diet. From these data they postulated the conversion of homocystine to methionine. Choline was found to be responsible for this conversion (du. Vigneaud et al., 1939), and it was further hypothesized by Simmonds et al. (1943) that a methyl group from choline was transferred in the formation of methionine. It has since been established that all of the methyl groups of choline were derived from methionine by way of transmethylation (Bremer and Greenberg, 1960).

Langer and Kratzer (1964) used poults from breeding stock which had been fed a practical ration and subjected them to a diet deficient in labile methyl groups. These workers used dimethylethanolamine to create the deficiency. On these deficient diets they found growth to be only 54

percent of that exhibited by poults fed an adequate dietary regime containing vitamin B_{12}, choline, and methionine (4.5 g/Kg). Growth improved in the presence of Vitamin B_{12} but was still only 65 percent of the control diet. They suggested that poults required vitamin B_{12} to synthesize methyl groups but this synthesis was inadequate to support optimum growth.

It was reported by Machlin et al. (1954) that chicken feathers have a higher amount of cystine than methionine and they suggested that this indicated the formation of cystine from dietary methionine.

Along this same line Block and Weise (1956) stated it was logical to assume that very young fowl are required to divert large proportions of their dietary sulfur containing amino acids to growth of feathers.

Rasheed and Oldfield (1964) removed two-thirds of the wings and the tails of chickens and found that normal chickens fed the supplemental diet gained faster and were more efficient than those on the deficient diet. Among the clipped chickens, methionine deficiency did not significantly affect growth or feed efficiency. At the same time normal chicks made better gains than clipped ones regardless of type of diet.

It has been noted that superoptimum levels of methionine result in growth depression and/or reduced feed efficiency. Tipton et al. (1965), while conducting an experiment comparing methionine to methionine hydroxy

analogue in a semi-purified diet containing 0.09 percent cystine, observed a growth depression with 1.04 percent total methionine. However, no change in feed efficiency was noted.

The ability of certain cells to concentrate glycine has been observed to be reduced when other amino acids including methionine were fed (Christensen, 1962). The higher the plasma level of these amino acids the more severe was the effect. Christensen suggested competitive inhibition was occurring among the amino acids. He also stated that the observations clearly indicated the concentration of the various amino acids did not proceed independently for each amino acid. It appeared logical to assume, if protein synthesis was to proceed normally, that there must be a requirement for every amino acid to be present at some limiting concentration. He concluded that when a large concentration of one amino acid accumulated, the ability of the cell to retain other amino acids was decreased.

Marrett et al. (1964) tested the growth of chicks by utilizing all-amino-acids-diets which contained no methionine or cystine. They added D,-L,- or DL-methionine at a 0.3 percent level and reported no significant improvement of any one over the other. At 0.5 percent the L enantiomorph significantly improved the growth of chicks as compared to the other two forms. All three forms were equivalent at 0.8 percent supplementation. The diet contained 3.8 percent D-amino acids which, according to the investigators, may have inhibited the utilization of D- and DL-methionine.

However, Almquist (1965) has suggested that a rate factor may be involved. He concluded that due to the time required for inversion of the D form it may be a less intense source and therefore show less of an inhibitory effect.

Kamath and Berg (1964) fed singly half-optimum levels of each of the L and D forms of all the readily invertible amino acids except arginine. These were fed in diets which had the DL form of the less readily invertible amino acids at two times the level of the L enantiomorph. These workers noted retardation in growth in varying amounts. The growth depression which resulted from D-methionine and D-phenylalanine was approximately the same, D-tryptophan gave a more marked depression, and D-histidine resulted in the greatest growth depression. These workers proposed that, in the presence of large amounts of poorly invertible D-amino acids, the readily invertible amino acids present in the largest concentration would compete more efficiently for the particular enzyme system. They based this conclusion on some earlier work by Bender and Krebs (1956), who made it clear that D-amino acid oxidase attacks some D-amino acids much faster than others. They reported D-methionine was oxidized by D-amino acid oxidase from sheep kidney twice as fast as D-tryptophan, three times as rapidly as D-phenylalanine, and thirteen times as rapidly as D-histidine.

Gordon and Sizer (1955) reported that chickens do not utilize D-methionine as completely as L-methionine and it

was postulated that this was because D-methionine was metabolized primarily in the kidney tissue and was partially lost by excretion. Later work by Fell et al. (1959) disagreed with these workers. They showed the isomers of methionine were equivalent in the chicken as judged by both nutritional response and nitrogen retention.

In the same year Berg (1959), announced that D-lysine, D-isoleucine, and D-threonine were inadequate for growth when compared to their L-isomer. However, D-methionine was observed to be as effective as L-methionine.

Wretland (1952) found the isomers of methionine to be equivalent if fed in an all-L-amino acid diet. Bauriedel (1963) postulated that this might be explained in terms of an overloading of the capacity of the chick to transform D-amino acids to useful metabolites. Therefore, Bauriedel (1963) postulated it was possible that a high level of D-methionine in the diet of chicks would result in a mistaken idea of the capacity of the chick to perform the necessary conversion.

The growth response obtained by supplementation with methionine hydroxy analogue was found by Bird (1952) to be equivalent to that obtained with DL-methionine when chicks are fed methionine deficient diets containing at least 20 percent protein. Some later work by Machlin and Gordon (1959) confirmed Bird's work. These investigators found no difference between methionine hydroxy analogue and methionine on a 12 percent protein diet.

However, Sullivan and Bird (1957) reported just the opposite in 12-14 percent protein diets for chicks. They observed that methionine hydroxy analogue failed to support performance equivalent to DL-methionine. This work was in agreement with Tipton et al. (1965) who reported that the analogue consistently failed to support growth equivalent to DL-methionine.

Kamin and Handler (1952) did not notice extremely specific competition among amino acid transport systems, although the rate of intestinal absorption of one amino acid was reduced in the presence of another amino acid. In this experiment the natural isomers were used and the neutral amino acids were administered in the isoelectric form, dicarboxylic amino acids as monosodium salts, and basic amino acids as the monohydrochlorides. The greatest degree of inhibition observed was approximately 50 percent. These data indicated three paths of absorption: one mechanism for the neutral, one for the acidic, and one for the basic amino acids.

Later work by Wiseman (1955) supported these data. He found with in vitro techniques that methionine was capable of inhibiting the absorption of many other amino acids from the intestine.

Jervis and Smyth (1959) observed that L-histidine and L-methionine were absorbed at similar rates and both were absorbed faster than the D isomer. D-methionine was absorbed faster than D-histidine. Furthermore, they reported that while D-histidine had no effect on D-methionine, the reverse

situation resulted in inhibition. Also, they reported L-methionine had a great effect on the absorption of D-histidine.

It was originally believed that D-amino acid transport was passive but Christensen et al. (1962) suggested a carrier system of low specificity might account for transfer of both L- and D-amino acids. The possibility that some D-amino acids were converted to the L-form during absorption was also proposed. The inhibition of D-histidine absorption by L-methionine (Jervis, 1959) might be explained on this basis although it had been previously reported by Gibson et al. (1954) that the capacity for synthesis of some L-amino acids from the corresponding D enantiomorphs was present only to a limited extent. However, histidine was not one of the amino acids tested.

Further information relating to this subject was supplied by Matthews and Smyth (1954). They established that D-amino acids were located in the portal blood after absorption from the lumen of the intestine. Therefore, they concluded that all D-amino acid transfer did not proceed by way of conversion to the L-form of the amino acid.

Oxender and Christensen (1963) observed that competition among neutral amino acids was quite widespread in Ehrlich cells. They proposed the view that these amino acids might represent a single transport unit which moved across the plasma membrane mediated by a single carrier.

The adaptive response of liver cells to prolonged

protein depletion followed by repletion was studied by Williams (1964). It was observed that methionine was capable of protecting the liver protein in a protein free ration and cystine and methionine significantly preserved the cells of the liver against loss of succinic oxidase and succinic dehydrogenase.

It has been reported that methionine functions as an essential nutrient in poultry rations and that supplementation with this material will, under certain circumstances, affect weight gain and feed conversion. It has also been reported that methionine is involved in one-carbon metabolism and that it is absorbed from the intestine mediated by some type of carrier mechanism. However, it has not been demonstrated which if any of these actions predominates at a particular level of supplementation.

EXPERIMENTAL PROCEDURES

Straight run broiler type chicks, obtained from a commercial hatchery, were used. The day-old chicks were weighed individually and only chicks which weighed between 43 and 46 grams were used. These chicks were wing banded at random, weighed and distributed into experimental lots of ten chicks each. Feed and water were supplied ad libitum throughout the experimental period of four weeks.

The chicks were confined in five-deck battery brooders equipped with thermostatically controlled electric heaters, metal dropping pans, and raised wire floors. Each deck was divided into two pens of ten chicks each. The study was conducted in an air-conditioned room equipped with horizontal fluorescent lighting.

A randomized block type design was used with the stipulation that the same treatment could appear in the same battery or deck only once in the trial. Three replications per treatment were used and statistical analyses of these data in this study were conducted as outlined by Steel and Torrie (1960).

In this trial four-week feed and body weights were recorded and two chicks from each pen were taken at random for further analysis. The basal ration used in this trial

is shown in Table I and the composition of the experimental diets in presented in Table II.

One chick from each pen (three per treatment) was orally dosed with 50 microcuries of C^{14} methyl labeled DL-methionine. The remaining chick which had been taken from each pen for analysis was given an oral dose of 50 microcuries of DL-methionine-2-C^{14}. These chicks were then confined for four hours in unheated finishing batteries surrounded by a polyethylene covering. This apparatus was equipped with fluorescent lighting and exhaust port for removal of any radioactive $C^{14}O_2$. Feed and water were supplied ad libitum during this period in an attempt to maintain metabolism as nearly identical to the growing period as was possible.

At the end of four hours a blood sample was taken from each chick by heart puncture and all the chicks were sacrificed. The organs removed for further study were: liver, kidney and approximately one-half of the pectoralis minor muscle from the right side of the chick.

The amount of radioactive material contained within one ml. of blood was immediately determined by the procedure of Smith et al. (1964). The liver, kidney, and muscle were chilled, weighed and homogenized in two volumes of cold 0.25 M sucrose in an Omni-mixer. This was followed by further homogenization with a Potter-Elvehjem type homogenizer. In the latter homogenization the pestle of the homogenizer was rotated at 600 to 1000 rpm by means of a stirring motor. The

TABLE I

COMPOSITION OF BASAL RATION

Ingredients	
Sucrose	48.57
Isolated soybean protein[a]	15.00
Gelatin	15.00
Corn oil	12.00
Ground cellulose (alphacel)	1.00
Terramycin (TMIO)	0.02
Vitamin A mix (30,000 I.U./gm.)	0.03
Vitamin D_3 mix (7,500 I.C.U./gm.)	0.02
Butylated hydroxy toluene	0.01
L-tryptophan	0.15
L-phenylalanine	0.20
Choline chloride (70%)	0.30
Vitamin premix[b]	0.50
Mineral mix[c]	6.00
Total	98.80
Calculated analysis:	
Crude protein (5)	26.48
Energy (prod Cal./kg.)	2626.00
C/P ratio	99.50
Methionine (5)	0.24
Cystine (%)	0.09
Lysine (%)	1.47
Choline chloride (mg./kg.)	1995.00

[a]Assay protein C-1, Skidmore Enterprise, Cincinnati, Ohio.

[b]Furnished in mg. per Kg of finished ration: vitamin B_{12}, 0.03; biotin, 0.30; menadione, 1.0; pyridoxine·HCl, 8.0; folic acid, 4.0; riboflavin, 16.0; nicotinic acid, 100.0 calcium pantothenate, 20.0; thiamin·HCl, 24.0.

[c]Furnished in gms/Kg. of ration: $CaCO_3$, 15; K_2HPO_4, 9; Na_2HPO_4, 7.3; $Ca_3(PO_4)_2$, 14; NaCl, 8.8; $MgSO_4 \cdot 7H_2O$, 5; ferric citrate, 0.4; $MnSO_4 \cdot 4H_2O$, 0.42; KI, 0.04; $ZnCo_3$, 0.02; $CuSO_4 \cdot 5H_2O$, 0.02.

TABLE II

RATIONS USED IN TRIAL I

Diet	Basal %	DL-Methionine %	Sucrose %	Total Methionine Content
1	98.80	--	1.20	0.24
2	98.80	0.30	0.90	0.54
3	98.80	0.60	0.60	0.84
4	98.80	0.90	0.30	1.14
5	98.80	1.20	--	1.44

test tube was moved up and down over the pestle to insure a high percentage of disrupted cells.

After homogenizing the organs, the liver was further fractionated into a nuclear fraction, a mitochondrial fraction, a microsomal fraction, and a soluble protein fraction using differential centrifugation. The isolation of the nuclear fraction was accomplished by layering three ml. of the liver homogenate carefully over seven ml. of cold 0.34 M sucrose which was contained in a 200 ml. cellulose mitrate tube. This preparation was centrifuged for 10 minutes at 2400 rpm (700 x g). All centrifugations were carried out in the Servall refrigerated centrifuge model RC-2 with angle rotor SS-34. The supernatant was removed with a capillary pipette and the sediment was resuspended in two ml. of cold 0.25 M sucrose and centrifuged again at the same speed for the same period of time. The supernatant was decanted and the precipitate was extracted from the centrifugation tubes and suspended in five ml. of 0.25 M sucrose. This fraction of the liver was referred to as the nuclear fraction although it was not completely cytologically homogeneous. The supernatant from the first separation was transferred to a 200 ml. cellulose nitrate tube and centrifuged for 10 minutes at 6500 rpm (5000 x g). The supernatant was removed along with the pink, partially segmented layer of particles found above the firmly packed fraction referred to here as the mitochondrial fraction. The mitochondrial fraction was then suspended in five ml. of 0.25 M sucrose.

The supernatant was transferred and the procedure repeated, as previously stated, with the exception that the centrifugation was accomplished at 14,000 rpm (24,000 x g).

The supernatants obtained from the above centrifugations were combined, brought to a total volume of 35 ml. with 0.25 M sucrose and centrifuged for 1.5 hours at 17,500 rpm (39,000 x g). The sediment resulting from this procedure was re-suspended in five ml. of 0.25 M sucrose and referred to as the microsomal fraction. The supernatant was regarded as the soluble protein fraction. All of these liver fractions plus the homogenate of muscle and kidney were placed in a freezer at -10°C.

Sample Combustion

The frozen homogenates of the muscle and kidney as well as the subcellular fractions of the liver were allowed to thaw and one ml. aliquots were placed into glass cups 25 mm. deep and eight mm. in diameter. These samples were immediately frozen at -10°C. The procedure of Smith et al. (1964) was used to determine the amount of radioactivity contained within the various samples.

Amino Acid Analyses

Ion exchange chromatography was used for the separation and determination of the amino acid content of the diets employed in this trial and the amino acid content of the soluble protein fraction of the liver [Moore et al. (1958), Moore and Stein (1951), Moore and Stein (1954), and

Spackman et al. (1958)]. Standards were run at various intervals to permit accurate calculations of the ninhydrin reactive materials.

Seventy-five mg. samples of the diets were hydrolyzed for six hours at 110°C. in vacuo in two mls. of 6 N HCl. Before hydrolysis 0.9 millimoles of cystine were added to each of these samples. It was ascertained that hydrolysis of crystalline amino acid mixtures resulted in partial destruction of a number of amino acids. As much as 90 percent of the methionine of these samples as well as lesser amounts of other amino acids was destroyed unless a redox buffer such as cystine was added. It was thought that sufficient oxygen still remained to allow oxidation to proceed. These data will be discussed in greater detail in another section.

After the six-hour hydrolysis was completed the samples were filtered through filter paper, flash evaporated to approximately one ml. volume, rinsed with 8-10 ml. of water and again flash evaporated to a volume of one ml. These samples were adjusted to approximately pH 7 with 2 N NaOH and 0.4 ml. of sodium sulfite (6.3 gm/100 ml.) added. After four hours had elapsed, the samples were pipetted into a 10 ml. volumetric flask and brought to volume with a citrate buffer of pH 2.2. One ml. of this material was placed on the amino acid analyzing column and the amino acid composition determined.

A composite sample was made of the soluble protein

fraction of the livers from the three chickens per diet which had been dosed with the methyl labeled methionine. This procedure was also followed for the soluble protein fractions from the three chickens which received the methionine labeled in the number two position. The amino acid composition of the combined samples as well as the amount of radioactivity in each individual amino acid were determined.

The sample was prepared for ion exchange chromatography by first evaporating the soluble protein fractions to approximately 15 ml. volume with a flash evaporator. This sample was transferred to a 200 ml. cellulose nitrate tube and 10 ml. of one percent picric acid were added. This mixture was centrifuged at 0°C. and 14,000 rpm (24,000 x g) for 10 minutes. The supernatant from this centrifugation was referred to as the soluble amino acid fraction. The precipitate, referred to as the protein bound amino acid, was left in the tube and stored in a -10°C. freezer. The supernatant was poured through a 15 mm. column of Dowex 2x8 resin which had been prepared by allowing 10 mls. of 1 N HCl to percolate through it. The acid treatment was followed by water until the effluent was approximately pH 6. The supernatant which was poured through this column was collected in a 500 ml. round-bottom boiling flask along with 20 ml. of 0.1 N HCl which was used to rinse the column. The boiling flask containing the sample was attached to a flash evaporating system and the volume reduced to approximately three ml. The

boiling flask was then rinsed with 10 ml. of water and evaporated until approximately 2.0 ml. remained in the flask. The sample was adjusted with 2 N NaOH until it was approximately pH 7, and 0.4 ml. of sodium sulfite (6.3 grams/100 ml.) was added. This mixture was allowed to stand four hours and adjusted to approximately pH 2 with 1 N HCl. The sample was transferred to a 10 ml. volumetric flask and brought to volume with a citrate buffer of pH 2.2. This sample was analyzed by ion exchange chromatography.

The effluent from this analysis was collected in five ml. portions by the use of a fraction collector and test tubes. The test tubes which contained portions of the separated amino acids were removed, and the amount of radioactivity contained within each individual amino acid was determined by the procedure already outlined.

The procedure for determination of the protein bound amino acids is as follows: the sample of precipitated material remaining within the cellulose nitrate tube after the centrifugation was removed, placed in a 500 ml. round-bottom boiling flask with 50 micrograms of cystine, and 10 ml. of 6 N HCl were added. To this was attached a stop-cock with a ground glass fitting, and this was secured by use of a clamp. The sample was placed under vacuum and put into a convection oven at 110°C. for 22 hours. The sample was then removed, filtered through filter paper and the procedure continued exactly as described for the soluble amino acid.

RESULTS AND DISCUSSIONS

The purpose of this experiment was to study the metabolism of chickens as affected by various levels of methionine ranging from below to well above the established requirement for growth and feed conversion.

The criteria employed were weight gain, feed conversion, and the amount of radioactivity found within various organs.

Weight Gain and Feed Utilization

A summation of the results for weight gain is shown in Table III. A preliminary analysis of variance indicated a highly significant difference in four-week weight gain and a breakdown of the degrees of freedom for each component was accomplished by orthogonal comparisons. These results are shown in Table IV. These analyses clearly revealed that the two lower levels of methionine (0.24 and 0.54 percent) were inadequate to support optimum weight gain under the conditions of this experiment.

The average weight gain of the chickens fed the 0.84 percent methionine ration was the highest with higher levels of methionine showing a progressive decline in weight gain. However, the mean weight gain of chicks receiving the 0.84

TABLE III

FOUR-WEEK WEIGHT GAIN

Diet Number	Percent Methionine	Weight Gain (Grams)
1	0.24	75.3± 5.86[a]
2	0.54	341.8±15.73
3	0.84	423.3± 6.30
4	1.14	397.9± 2.56
5	1.44	392.9±24.54

[a]Mean and standard error.

TABLE IV

ANALYSIS OF VARIANCE

Orthogonal Comparison for Treatment
Four-Week Weight Gain

Source	D.F.	M.S.	F
Level of methionine[a]	4	61,671	134.36[b]
(1) 1 vs. 2, 3, 4, and 5	1	236,585	515.43[b]
(2) 2 vs. 3, 4, and 5	1	8,991	19.59[b]
(3) 3 vs. 4 and 5	1	1,664	3.62
(4) 4 vs. 5	1	38	0.08
Error	8	459	

[a]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 per cent.

[b]Significant at the 0.01 level of probability.

percent methionine diet was not statistically different from the ones receiving the two higher levels of methionine.

These data are generally in agreement with Tipton et al. (1965) who reported that a total methionine level of 1.04 percent was sufficient to produce a statistically significant growth depression. In these studies dietary levels equal to or greater than 1.14 percent resulted in a depression in growth; however, this depression was nonsignificant. It is felt that the variation encountered in this study may be responsible for the failure to achieve significance. However, these data follow the same trend as that reported by Tipton et al.

A summary of four-week feed conversions and grams of methionine consumed per chick day are shown in Table V.

The analysis of variance for feed conversion showed a significant treatment effect at the 0.01 level of probability. An increase in dietary methionine from 0.24 to 0.54 percent and from 0.54 to 0.84 percent resulted in a significant improvement in feed utilization as illustrated by the orthogonal comparisons which were conducted (Table VI).

Dietary methionine levels above 0.84 percent tended to improve feed consumption. However, the improvement was not statistically significant.

The analysis of variance of the grams of methionine consumed per chick day (Table VII), only reiterated the results of the weight gain and feed conversion. As these are a function of feed consumed, they were expected to increase

TABLE V

FOUR-WEEK FEED CONVERSION AND METHIONINE CONSUMPTION

Feed Conversion[a]	
Level of Methionine Percent	Feed Gain Grams
0.24	3.63±0.082[b]
0.54	1.81±0.038
0.84	1.52±0.035
1.14	1.44±0.090
1.44	1.45±0.120

[a]Feed consumed per gram gain.

[b]Mean and standard error.

Methionine Consumption[a]	
Level of Methionine Percent	Consumed Mgms.
0.24	23.6±2.6[b]
0.54	119.4±5.0
0.84	192.8±6.8
1.14	234.8±15.5
1.44	304.9±28.6

[a]Milligrams consumed per chick per day.

[b]Means and standard error.

TABLE VI

ANALYSIS OF VARIANCE OF FOUR-WEEK FEED CONVERSION

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[a]	4	26.43	112.47[b]
(1) 1 vs. 2, 3, 4, and 5	1	103.30	439.57[b]
(2) 2 vs. 3, 4, and 5	1	2.60	11.06[c]
(3) 3 vs. 4 and 5	1	1.12	0.48
(4) 4 vs. 5	1	0.01	0.01
Error	8	0.24	

[a]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[b]Significant at the 0.01 level of probability.

[c]Significant at the 0.05 level of probability.

TABLE VII

ANALYSIS OF VARIANCE OF METHIONINE CONSUMPTION

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[a]	4	35.08	35.98[b]
(1) 1 vs. 2, 3, 4, and 5	1	86.00	95.55[b]
(2) 2 vs. 3, 4, and 5	1	35.00	38.88[b]
(3) 3 vs. 4 and 5	1	11.80	13.11[b]
(4) 4 vs. 5	1	7.30	8.11[c]
Error	8	0.90	

[a]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[b]Significant at the 0.01 level of probability.

[c]Significant at the 0.05 level of probability.

proportionally.

In Table VIII the total feed consumed per diet for the four-week experimental period as well as the grams of weight gain per gram of methionine consumed is presented. Although it is well known that energy consumption is the primary regulatory mechanism for feed consumption it can be clearly seen that amino acid deficiencies (0.24 percent level) will significantly depress feed consumption. At the other extreme of the dietary regimes feed consumption was again depressed. This was attributed to an amino acid imbalance and may actually be an expression of an overloaded transport mechanism. This would again clearly demonstrate that amino acid balance and content are more important than the actual percent protein in the diet. It is also significant to note that, as the amount of methionine in the diet increased, the efficiency of utilization decreased.

The preceding data have led the author to postulate that nutrient requirements should be associated with actual intake as opposed to simply stating them as percent of the diet. This could easily be accomplished by relating all nutrient requirements to energy consumption.

In this fashion excesses as well as deficiencies could be avoided.

TISSUE ANALYSIS

As was mentioned in an earlier section, the livers, kidneys, and approximately one-half of the pectoralis minor

TABLE VIII

FEED AND METHIONINE CONSUMPTION

Dietary Methionine Levels	Feed Consumption	Methionine[a] Efficiency
Percent	Grams	
0.24	272.3	114.8
0.54	619.0	102.3
0.84	643.0	78.3
1.14	573.1	60.9
1.44	569.9	47.9

[a]Grams gain in body weight per gram of methionine consumed.

muscle from the right side of the chick were removed for analysis. It was thought that these analyses might provide further information as to the dual biological mechanisms of methionine--protein synthesis and transmethylation.

It is an established fact that methionine functions as an integral part of both of these mechanisms, and this study was planned to try to determine if a change in the dietary level of methionine would result in the chicken utilizing this material preferentially for one or the other of these functions.

Muscle Tissue

The amount of radioactivity recovered from muscle tissue is shown in Table IX. The values are expressed as percent of the total methionine dose recovered per gram of muscle. The lowest level of dietary methionine (0.24 percent) was found to have the highest percent recovery of labeled carbon, and analysis of variance (Tables X and XI) revealed a highly significant difference that could be attributed to the dietary levels of methionine. A breakdown of the degrees of freedom by orthogonal comparisons showed this difference to be between the 0.24 percent dietary methionine level and the other four levels, indicating a dietary level of 0.54 percent or above was adequate for protein synthesis in muscle tissue as protein synthesis is defined in this section. This was noted regardless of whether the muscle was obtained from a chick which had received the methionine-2-C^{14} or the

TABLE IX

RECOVERY OF RADIOACTIVITY FROM MUSCLE TISSUE[a]

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	1.09±0.24	4.60±0.31[b]
0.54	0.27±0.01	0.50±0.16
0.84	0.18±0.01	0.68±0.12
1.14	0.16±0.01	0.40±0.03
1.44	0.40±0.02	0.59±0.10

[a]Data expressed as recovery of total dose administered per gram of muscle tissue.

[b]Mean and standard error.

TABLE X

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM MUSCLE TISSUE[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	45.03	18.30[c]
(1) 1 vs. 2, 3, 4, and 5	1	169.80	69.02[c]
(2) 2 vs. 3, 4, and 5	1	0.08	0.03
(3) 3 vs. 4 and 5	1	1.84	1.30
(4) 4 vs. 5	1	8.21	4.75
Error	8	2.46	

[a]Methionine labeled in the methyl carbon.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

TABLE XI

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM MUSCLE TISSUE[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	98.85	99.55[c]
(1) 1 vs. 2, 3, 4, and 5	1	394.00	396.78[c]
(2) 2 vs. 3, 4, and 5	1	0.07	0.07
(3) 3 vs. 4 and 5	1	0.71	0.72
(4) 4 vs. 5	1	0.52	0.53
Error	8	0.99	

[a]Methionine labeled in the number two position.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

$C^{14}H_3$ labeled methionine.

It should be mentioned that the precent recovery of labeled carbon of methionine-2-C^{14} was much greater than the percent recovery of labeled carbon from the more labile methyl group of methionine. In certain cases there was as much as a four-fold difference in the percent recovery of the two isotopically labeled carbons. This was not surprising because the labile methyl group of $C^{14}H_3$ methionine was expected to be distributed much more widely than the carbon chain of the methionine-2-C^{14}. The labeled carbon of methionine-2-C^{14} was assumed to remain with the intact molecule or at least with the portion of the molecule remaining after transmethylation. These data did, however, indicate that rapid transmethylation was occurring at all levels of dietary methionine and in particular at the lowest level (0.24). If transmethylation was not proceeding at this dietary level, the percentage recoveries of isotopically labeled carbon from the two positions of methionine would have been equivalent. This would tend to refute the postulation which proposes a preferential use of inadequate levels of methionine for protein synthesis.

It was previously noted that maximum weight gain was obtained at a level of 0.84 percent dietary methionine and it was found that maximum utilization of methionine for protein synthesis in the muscle occurred at or below the 0.54 percent level.

Although the ratio between the percent recovery of

labeled carbons from the two positions of radioactivity in the methionine molecules (0.24 percent level) was four to one, it was noted that the ratio between the percent recovery of the labeled material of methionine-2-C^{14} and $C^{14}H_3$ methionine was never less than approximately two to one, even at the highest levels of supplementation. Therefore, one might postulate that a chicken supplied with an inadequate amount of methionine would satisfy its methionine requirement for protein synthesis before it satisfied anything but the very minimum requirement for one-carbon metabolism. If both transmethylation and protein synthesis are required for maximum anabolism, this then could explain why maximum weight gain was obtained at a higher level of dietary methionine than was maximum protein synthesis in the muscle. These data could indicate that 0.54 percent dietary methionine would supply the requirement for protein synthesis but that a greater amount (0.84 percent) was required to satisfy the requirement for both protein synthesis and transmethylation. This of course, would indicate that protein formation would take preference over transmethylation functions in muscle tissue supplied with inadequate amounts of methionine; however, it should again be pointed out that rapid transmethylation was occurring even at the lowest level of methionine supplementation (0.24 percent).

Kidney Analysis

The summation of the kidney data is found in Table XII.

TABLE XII

RECOVERY OF RADIOACTIVITY FROM TOTAL KIDNEY[a]

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	4.77±0.11[b]	6.63±0.17[b]
0.54	3.33±0.32	10.20±1.17
0.84	3.27±0.14	5.48±0.80
1.14	3.93±0.36	5.28±0.68
1.44	2.31±0.21	5.97±1.17

[a]Data expressed as percent recovery of radioactivity of total kidney.

[b]Mean and standard error.

It is obvious that the statistical difference in recovery of the methionine-2-C^{14} is centered around the 0.54 percent level of dietary methionine which resulted in a higher recovery value of the labeled carbon. This might be explained on this basis; if, at the low levels of methionine supplementation, the kidney was concentrating the amino acid in order to conserve the limited amount which was supplied and, if a large amount of transmethylation had previously occurred at the 0.24 percent level, then the percent recovery of the $C^{14}H_3$ methionine possibly might not indicate any difference at the 0.54 percent level. This would be because a certain amount of the labeled carbon would have already been removed and concentration of the remaining material could still indicate a low value. However, in the molecule labeled in the number two position, transmethylation would have no effect because the labeled carbon would remain within the molecule. Therefore, any concentration of the 0.54 percent dietary level of methionine by the kidney would result in a much higher recovery of the methionine-2-C^{14}. This interpretation would suggest that transmethylation was occurring to a substantial extent at the 0.24 percent dietary level of methionine.

The analysis of variance of the recovery of the $C^{14}H_3$ methionine from the kidney is shown in Table XIII. A further division of the degrees of freedom by orthogonal comparisons is also shown in Table XIII. A highly significant difference was noted between the 0.24 percent level of methionine and

TABLE XIII

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM TOTAL KIDNEY[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	24.82	11.36[c]
(1) 1 vs. 2, 3, 4, and 5	1	58.47	26.77[c]
(2) 2 vs. 3, 4, and 5	1	0.58	0.26
(3) 3 vs. 4 and 5	1	0.45	0.21
(4) 4 vs. 5	1	39.40	18.04[c]
Error	8	2.18	

[a]Methionine labeled in the methyl carbon.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

all other treatments and between the 1.14 and 1.44 percent levels. The difference between the 0.24 percent level and all other levels only reiterates the statements made previously. The fact that the 1.44 percent level of methionine was significantly lower than the 1.14 percent level could well indicate that selective reabsorption was proceeding less rapidly than when the chick was receiving a diet lower in methionine.

The percent recovery of the number two labeled methionine from the kidney was analyzed (Table XIV). This analysis revealed that the chicks receiving the 0.54 percent methionine appeared to utilize it differently than did the chicks receiving the remaining levels. The mean recovery value for the 0.54 dietary level was higher than the means of the other dietary treatments and this value, as determined by orthogonal comparisons, was different to a highly significant degree.

The basic observation throughout this phase of the experiment was that a dietary methionine level of 0.84 percent was required for maximum weight gain and feed conversion. However, isotopically labeled carbon analyses of muscle tissue indicated that a dietary level of 0.54 percent dietary methionine resulted in maximum utilization and apparently maximum protein formation. It further appears that both protein syntheses and transmethylation by methionine are required for maximum weight gain and feed conversion.

From these data no definite conclusions were drawn as

TABLE XIV

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM TOTAL KIDNEY[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	12.21	4.67[c]
(1) 1 vs. 2, 3, 4, and 5	1	0.02	0.01
(2) 2 vs. 3, 4, and 5	1	48.09	18.35[d]
(3) 3 vs. 4 and 5	1	0.04	0.02
(4) 4 vs. 5	1	0.71	0.27
Error	8		

[a]Methionine labeled in number two position.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.05 level of probability.

[d]Significant at the 0.01 level of probability.

to a preferential use of methionine for protein synthesis or one-carbon metabolism in the two lower dietary levels. It is possible that there is no preference and that these two functions operate simultaneously regardless of the dietary level of methionine. Above a dietary level of 0.84 percent methionine it appears that a steady state has been achieved because the ratio of the percent recovery values did not vary to any significant extent. It was noted, however, that the percent recovery of methionine-2-C^{14} remained slightly higher than the $C^{14}H_3$ methionine even at these higher dietary levels.

Blood Analyses

A sample of blood was taken from the chicks immediately before they were sacrificed. This was done because it was thought that these data might reveal significant information relating to absorption from the intestinal tract.

A summation of these data is shown in Table XV. An analysis of variance indicated that there were no differences between the treatment means. This could well be attributed to the extreme variation of the means. It is believed that the blood levels were only a function of the amount and constancy of a chick's feeding habits. However, since there is no way of ascertaining this information at this time a definite conclusion could not be drawn.

TABLE XV

RECOVERY OF RADIOACTIVITY FROM BLOOD[a]

Dietary Level of Methionine Percent	Recovery of Dose of $C^{14}H_3$ Methionine Percent	Recovery of Dose of No. 2 Labeled Methionine Percent
0.24	0.12±0.04	0.22±0.28[b]
0.54	0.04±0.01	0.12±0.04
0.84	0.04±0.02	0.05±0.04
1.14	0.06±0.02	0.11±0.02
1.44	0.07±0.02	0.08±0.05

[a]Data expressed as recovery of radioactivity on a per ml. basis.

[b]Mean and standard error.

Liver Analysis

The summation of the liver data is presented in Table XVI. At the 0.24 dietary level of methionine, approximately three-fourths of the activity administered as methionine-2-C^{14} was recovered. At the 0.54 percent level the recovery decreases to slightly over fifty percent. With higher levels of supplementation less than one-third of the activity is recovered and there were no significant differences noted between these means. This would indicate that a steady state had been established for protein formation.

If assumptions are made that the liver is the primary site of protein synthesis and that the incorporation of methionine-2-C^{14} is a valid indication of protein synthesis, then in Table XVII it can be seen that some protein synthesis is occurring at all dietary levels and the incorporation of methionine-2-C^{14} into protein reaches a maximum at the 0.84 percent dietary level. It can be seen that there was a progressive decline above this amount. When these data are considered in light of the muscle data which indicated that no improvement was noted above the 0.54 percent dietary level, it appears that conflicting data have been reported. However, it must be recognized that the liver is involved in a multitude of functions and so may easily be much more indicative of the physiological well-being or nutritional requirement of the animal than any other single organ or group of organs.

When one considers the labile methyl data in Table XVI, it is apparent that transmethylation is occurring at a

TABLE XVI

RECOVERY OF RADIOACTIVITY FROM TOTAL LIVER[a]

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	28.27±4.20[b]	73.53±7.65[b]
0.54	11.64±0.16	55.12±2.55
0.84	28.75±2.26	28.35±0.19
1.14	24.52±3.62	23.33±0.95
1.44	28.05±2.40	25.11±3.92

[a]Data expressed as percent recovery of radioactivity administered on a total liver basis.

[b]Mean and standard error.

TABLE XVII

METHIONINE-2-C^{14} INCORPORATION INTO PROTEIN

Level of Methionine	Recovery of Methionine-2-C^{14}	Gain per Gram Methionine Incorporated into Protein
Percent	Percent	
0.24	73.5	154.4
0.54	55.1	180.8
0.84	28.4	275.9
1.14	23.3	259.8
1.44	25.1	183.3

very rapid rate at both the 0.24 and the 0.54 levels of supplementation. These data also approach a steady state at 0.84 percent and above because these recovery values are in about a one-to-one ratio with that labeled in the number two position.

It was noted that the ratio of percent recovery of the number two labeled methionine to the $C^{14}H_3$ methionine became wider at the 0.54 level as compared to the ratio at the 0.24 level (5:1 vs. 2.5:1). From this data it might be inferred that at a level of 0.54 percent dietary methionine the requirement for protein synthesis would have been met and a much larger percentage of the amount of methionine available would then be utilized for transmethylation. However, as in the muscle, transmethylation was occurring at a rapid rate at the two lower dietary levels as measured by the ratios between the percent recovery for the two isotopically labeled materials. However, these data could be explained on the basis of rapid transmethylation at the 0.24 percent level of dietary methionine followed by extensive protein synthesis at the 0.54 percent dietary level. This would result in a wider ratio at the 0.54 percent dietary methionine because if rapid transmethylation had occurred at the 0.24 percent level, the percent recovery value of methionine-2-C^{14} would not have been affected and only the recovery value of the labile labeled carbon would have been decreased.

Further, if the 0.54 percent level supplied the organism with sufficient methionine to adequately supply the

transmethylation prior to protein formation then incorporation of methionine-2-C^{14} as well as the transmethylation vehicle (methionine which had lost the labeled carbon) would result in a widening of the ratio between the recovery values of the two tagged materials. This, of course, assumes that some transmethylation is occurring at all levels.

The analysis of variance for the liver are found in Tables XVIII and XIX. The analysis of the percent recovery of the methyl labeled methionine from the liver (Table XVIII) only approached significance (F value of 3.7 as compared to 3.8). However, orthogonal comparisons indicated a highly significant difference between the 0.54 percent level and the remaining levels of methionine. The value for 0.54 percent dietary level was approximately half as large as the percent recovery value of the other dietary levels. However, the author wishes to point out that these data appear somewhat unusual when they are plotted and there is a possibility that the differences which were noted were produced by chance alone and were not a result of dietary regime.

Orthogonal comparisons of the percent recovery of methionine-2-C^{14} from the liver (Table XIX) indicated that there was a highly significant difference between the lowest level of methionine (0.24 percent) and the remaining levels. The lowest dietary level of methionine (0.24) resulted in the highest percent recovery of labeled carbon. Another highly significant difference was between the 0.54 percent dietary level and the three higher levels. These data can

TABLE XVIII

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM TOTAL LIVER[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	157.51	3.72
(1) 1 vs. 2, 3, 4, and 5	1	60.72	1.43
(2) 2 vs. 3, 4, and 5	1	528.24	12.98[c]
(3) 3 vs. 4 and 5	1	12.15	0.29
(4) 4 vs. 5	1	30.84	0.71
Error	8	42.37	

[a]Methionine labeled in methyl carbon.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

TABLE XIX

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM TOTAL LIVER[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	1486.79	30.69[c]
(1) 1 vs. 2, 3, 4, and 5	1	3946.80	81.47[c]
(2) 2 vs. 3, 4, and 5	1	1961.16	40.48[c]
(3) 3 vs. 4 and 5	1	34.11	0.70
(4) 4 vs. 5	1	4.75	0.10
Error	8	48.45	

[a]Methionine labeled in the number two position.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

be explained on the basis that the two lowest levels of methionine (0.24 and 0.54) are inadequate and therefore uptake and utilization of the administered material would be a maximum.

LIVER FRACTIONATIONS

Prior discussion in this manuscript revealed that the liver was separated into four subfractions by differential centrifugation. These were designated as the nuclear, mitochondrial, microsomal, and soluble protein fractions. This procedure was incorporated into the experiment in order to determine more definitely the movement of the labeled methionine.

Nuclear Fractions

The mean percent recovery values of labeled carbon from the nuclear fractions are shown in Table XX. Analysis of variance revealed that there were no significant differences that could be attributed to varying the level of methionine. In that the nuclear portion of the cell is thought to be rather stable these data are surprising as appreciable incorporation had occurred.

Mitochondrial Fractions

Results similar to that reported for the nuclear fractions were noted for the mitochondrial fractions (Table XXI). The only exception was that the 1.14 percent dietary level of methionine resulted in a recovery value that was

TABLE XX

RECOVERY OF RADIOACTIVITY FROM NUCLEAR FRACTIONS[a]

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	3.43±0.10[b]	12.02±2.69[b]
0.54	2.79±0.38	9.23±0.31
0.84	3.33±0.76	4.11±0.46
1.14	4.73±0.44	6.33±1.89
1.44	3.83±0.58	6.54±0.46

[a]Data expressed as percent recovery of radioactivity on a total liver basis.

[b]Mean and standard error.

TABLE XXI

RECOVERY OF RADIOACTIVITY FROM MITOCHONDRIAL FRACTIONS[a]

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	0.83±0.14[b]	3.35±0.44[b]
0.54	0.86±0.15	3.67±0.58
0.84	0.78±0.05	2.31±0.48
1.14	1.61±0.11	2.01±0.32
1.44	0.49±0.04	1.89±0.28

[a]Data expressed as percent recovery of radioactivity on a total liver basis.

[b]Mean and standard error.

different ($P < 0.01$) from the 1.44 percent level (Table XXII). This was observed only in the percent recovery values from the $C^{14}H_3$ labeled methionine, and no differences were noted between the 1.14 and 1.44 percent dietary level of methionine in the data from the methionine-2-C^{14}. The explanation for this phenomenon was not readily apparent. However, since this fraction of the liver is not actively involved in amino acid metabolism it was thought that this was probably of no major importance.

TABLE XXII

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM MITOCHONDRIAL FRACTIONS[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	5.26	10.52[c]
(1) 1 vs. 2, 3, 4, and 5	1	2.77	0.55
(2) 2 vs. 3, 4, and 5	1	2.22	0.44
(3) 3 vs. 4 and 5	1	14.96	2.99
(4) 4 vs. 5	1	190.51	38.10[c]
Error	8	5.00	

[a]Methionine labeled in the methyl carbon.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

Microsomal Fractions

The percent of recovery of the radioactivity found in the microsomal and soluble protein fractions is presented in Tables XXIII and XXV. Statistical analyses and breakdown of degrees of freedom for these fractions are shown in Tables XXIV, XXVI, and XXVII.

The analysis of the percent recovery values of the methionine-2-C^{14} from the microsomal fraction revealed a significant difference ($P > 0.01$). The 0.54 percent dietary level of methionine was higher than the remaining levels.

Soluble Protein Fractions

In the soluble protein fraction there was a significant difference between the 0.54 percent methionine level and the higher levels of $C^{14}H_3$ methionine. Also, the recovery values for the methionine-2-C^{14} indicated a difference between the two low and the three high levels of methionine ($P > 0.01$). The three higher dietary levels of methionine resulted in a lower recovery of labeled carbon.

The examination of these data could indicate that active transmethylation was occurring. This was particularly noted in the data from the two lower levels of methionine. The percent recovery from the three higher levels of methionine suggest that a steady state between the methionine requirement for protein synthesis and one-carbon metabolism had been established. This would confirm previous observations made concerning the liver. The greatest difference

TABLE XXIII

RECOVERY OF RADIOACTIVITY FROM MICROSOMAL FRACTIONS[a]

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	1.34±0.15[b]	2.15±0.64[b]
0.54	0.50±0.18	3.04±0.54
0.84	1.26±0.13	0.78±0.01
1.14	0.74±0.09	1.05±0.14
1.44	1.15±0.34	1.21±0.20

[a]Data expressed as percent recovery of radioactivity on a total liver basis.

[b]Mean and standard error.

TABLE XXIV

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM MICROSOMAL FRACTIONS[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	26.24	7.72[c]
(1) 1 vs. 2, 3, 4, and 5	1	9.71	2.88
(2) 2 vs. 3, 4, and 5	1	92.29	27.39[c]
(3) 3 vs. 4 and 5	1	2.54	0.75
(4) 4 vs. 5	1	0.42	0.12
Error	8	3.37	

[a]Methionine labeled in the number two position.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

TABLE XXV

RECOVERY OF RADIOACTIVITY FROM SOLUBLE PROTEIN FRACTIONS[a]

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	16.12 ± 4.45[b]	21.64 ± 1.24[b]
0.54	6.97 ± 0.48	21.05 ± 2.74
0.84	17.24 ± 2.95	12.19 ± 0.50
1.14	13.57 ± 0.36	13.64 ± 0.51
1.44	11.77 ± 2.17	11.57 ± 1.95

[a]Data expressed as a percent recovery of radioactivity on a total liver basis.

[b]Mean and standard error.

TABLE XXVI

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM SOLUBLE PROTEIN FRACTIONS[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	49.39	2.16
(1) 1 vs. 2, 3, 4, and 5	1	36.54	1.59
(2) 2 vs. 3, 4, and 5	1	117.72	5.14[c]
(3) 3 vs. 4 and 5	1	41.77	1.82
(4) 4 vs. 5	1	4.86	0.21
Error	8	22.90	

[a]Methionine labeled in the methyl carbon.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.05 level of probability.

TABLE XXVII

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM SOLUBLE PROTEIN FRACTIONS[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	72.78	8.93[c]
(1) 1 vs. 2, 3, 4, and 5	1	118.53	14.55[c]
(2) 2 vs. 3, 4, and 5	1	165.77	20.35[c]
(3) 3 vs. 4 and 5	1	0.34	0.04
(4) 4 vs. 5	1	6.43	0.79
Error	8	8.15	

[a]Methionine labeled in the number two position.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

between the percent recovery of $C^{14}H_3$ methionine and methionine-2-C^{14} was centered around the 0.54 percent dietary level. In the microsomal and soluble protein fractions the amount of recovery of $C^{14}H_3$ methionine from the 0.54 level was approximately thirty percent of that recovered from the methionine-2-C^{14}.

This could be explained as meaning that, although transmethylation was occurring at a rapid rate under even the most deficient dietary regimes, the greatest amount of transmethylation was occurring at the 0.54 percent dietary methionine level. This might indicate that protein synthesis was predominant to transmethylation at the 0.24 level of methionine.

Another postulation could be that a large amount of transmethylation had occurred at the 0.24 percent dietary methionine level and that this was followed by extensive protein synthesis at the 0.54 level. This latter postulation could account for the low percent recovery noted from the 0.54 percent level of $C^{14}H_3$ methionine.

However, these data indicate that both functions are operative at all dietary levels and no apparent preference was noted.

Total Recovery of Labeled Carbon

In an attempt to detect any treatment differences which persisted throughout the entire experiment, the total amount of radioactivity obtained from all of the organs

studied was compiled and an over-all percent recovery value was obtained.

Values for the blood were calculated on the basis that the chick contained ten percent of its total weight as blood (Newell and Shaffner, 1950).

The muscle estimation was accomplished by assuming the total muscle content of the chick to be thirty-seven percent of the live weight (Johnson, unpublished data) and muscle deposition of the labeled material to be uniform throughout the body.

The means of these accumulated determinations are shown in Table XXVIII. The analyses of variance with a breakdown of degrees of freedom by orthogonal comparisons are presented in Tables XXIX and XXX. The analysis of the methyl labeled methionine (Table XXVIII) clearly indicates a reduced value at the 0.54 level of methionine. This difference is highly significant.

The statistical difference ($P > 0.01$) noted in the data of the methionine-2-C^{14} only reveals that the lower level of dietary methionine (0.24) was different from the remaining diets and that the second level (0.54) was statistically ($P > 0.01$) different from the last three (Table XXX). These data only repeat what has previously been discussed and therefore will not be restated here.

TABLE XXVIII

RECOVERY OF RADIOACTIVITY FROM ALL SOURCES

Dietary Level of Methionine	Recovery of Dose of $C^{14}H_3$ Methionine	Recovery of Dose of No. 2 Labeled Methionine
Percent	Percent	Percent
0.24	39.81±4.45[a]	105.32±8.60[a]
0.54	20.64±0.15	77.53±1.13
0.84	37.20±1.60	49.25±2.03
1.14	33.90±3.83	40.23±1.24
1.44	40.18±4.48	45.06±5.19

[a]Mean and standard error.

TABLE XXIX

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM ALL SOURCES[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	195.01	5.68[c]
(1) 1 vs. 2, 3, 4, and 5	1	92.26	2.68
(2) 2 vs. 3, 4, and 5	1	609.10	17.73[d]
(3) 3 vs. 4 and 5	1	0.05	.01
(4) 4 vs. 5	1	59.16	1.72
Error	8	34.35	

[a]Methionine labeled in the methyl carbon.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.05 level of probability.

[d]Significant at the 0.01 level of probability.

TABLE XXX

ANALYSIS OF VARIANCE OF RECOVERY OF RADIOACTIVITY FROM ALL SOURCES[a]

Orthogonal Comparison for Treatment

Source	D.F.	M.S.	F
Level of methionine[b]	4	2272.55	40.56[c]
(1) 1 vs. 2, 3, 4, and 5	1	6568.67	117.25[c]
(2) 2 vs. 3, 4, and 5	1	2403.45	42.90[c]
(3) 3 vs. 4 and 5	1	87.25	1.56
(4) 4 vs. 5	1	34.99	0.62
Error	8	56.02	

[a]Methionine labeled in the number two position.

[b]Levels of methionine: 0.24, 0.54, 0.84, 1.14, and 1.44 percent.

[c]Significant at the 0.01 level of probability.

AMINO ACID ANALYSIS

Composite samples of the soluble protein fractions were made and these were separated by ion exchange chromatography in an attempt to follow the path of the labeled carbon into other amino acids.

It has been previously mentioned that the addition of a redox buffer to a crystalline amino acid mixture which was to be hydrolyzed greatly facilitated quantitative recovery of the various amino acids. These data are presented in Table XXXI. They clearly indicated that a substantially larger portion of the individual amino acids was recovered when cystine was added to the mixture. It was noted that the methionine recovery from the sample which was devoid of cystine was only ten percent of the non-hydrolyzed sample. It was further evident that the breakdown products were ammonia and aspartic acid or at least compounds which were eluted simultaneously with these entities.

It was hypothesized that a redox buffer would be unnecessary when a protein sample was to be hydrolyzed. The underlying supposition here was that a protein sample would possess greater buffering ability than the amino acid mixture and thus protection of the amino acids would be accomplished. However, 50 micromoles of cystine were added to a precautionary measure. Later findings indicated that this was in fact necessary. It was noted that no cysine appeared in the effluent from the amino acid analyzer. From these data it

TABLE XXXI

ANALYSES OF AMINO ACID MIXTURES

Amino Acid	22 Hour Hydrolysis Percent Recovery	22 Hour Hydrolysis With Added Cystine Percent Recovery
Lysine	110	105
Histidine	101	148
Ammonia	179	100
Arginine	113	99
Aspartic acid	187	103
Threonine	82	99
Serine	84	102
Glutamic acid	86	96
Proline	90	111
Glycine	107	106
Alanine	90	101
Valine	81	102
Methionine	10	93
Isoleucine	84	102
Leucine	84	105
Tyrosine	82	104
Phenylalanine	91	105

was inferred that the cystine was indeed being oxidized regardless of the protein present in the sample.

The soluble protein fractions were subjected to amino acid analysis by ion exchange chromatography. These amino acid samples were converted into $C^{14}O_2$ and the amount of radioactivity determined in a scintillation counter. The results of these determinations are presented in Tables XXXII and XXXIII.

Considerable distribution of the labeled carbon atoms was noted and the majority of the labeled $C^{14}H_3$ methionine appeared in arginine, aspartic acid, threonine, serine, glutamic acid, methionine and to some extent in glycine.

The appearance of radioactivity in glycine and serine can be easily explained by the labile methyl group being transferred to tetrahydrofolic acid and then to glycine and then becoming the B-carbon of serine. This carbon can become the methyl carbon of cystine and of pyruvate. Therefore, alanine could easily be labeled from pyruvate and labeled pyruvate could enter the citric acid pathway by addition of CO_2 to form oxaloacetate and thus glutamate and aspartate could well be labeled. The appearance of labeling in most of the other amino acids could then be traced from intermediates of the citric acid cycle. The fact that lysine was labeled is of interest because lysine synthesis by the chick has never been established. The appearance of this label may have resulted by way of the formate pool or by a more direct pathway because labeled lysine was also recovered

TABLE XXXII

RECOVERY OF RADIOACTIVITY FROM $C^{14}H_3$ METHIONINE[a]

	Diet 1		Diet 2		Diet 3		Diet 4		Diet 5	
	S	P	S	P	S	P	S	P	S	P
Lysine	T	6.81	T		T	5.12	T	1.51	5.10	3.51
Histidine	5.55	5.63	T		T	T	T	2.42	T	3.51
Arginine	5.20	7.26	20.80		T	T	8.77	6.80	1.42	13.16
Aspartic Acid	10.98	8.16	29.13		5.68	22.85	8.77	3.63	11.33	4.39
Threonine	10.98	3.63	9.89		3.19	15.23	4.38	3.63	11.05	4.39
Serine	10.46	3.63	2.72		47.47	10.15	46.79	10.88	11.05	9.06
Glutamic Acid	27.89	8.16	29.13		4.78	5.71	17.54	12.70	11.05	9.06
Proline	7.84	1.36	T		5.86	T	T	11.64	8.50	8.77
Glycine	6.27	9.07	1.56		6.58	T	T	1.81	11.05	10.53
Alanine	3.13	7.26	T		T	T	4.67	1.36	5.95	2.34
Cysteine	T	T	3.12		14.42	T	T	T	T	T
Valine	4.70	3.63	T		T	5.71	T	T	T	7.03
Methionine	T	21.78	T		12.01	20.62	T	25.39	4.25	5.40
Isoleucine	2.08	3.63	T		T	3.17	1.40	3.63	3.40	1.46
Leucine	1.74	3.63	3.12		T	2.54	1.20	T	5.66	5.26
Tyrosine	T	2.27	T		T	5.08	4.67	4.23	2.83	4.39
Phenylalanine	3.17	4.08	T		T	3.81	T	0.68	6.80	7.16
B alanine	T	T	T		T	T	T	T	T	T

[a]Numbers represent a percent of the total radioactivity recovered from each analysis.

T = 1 percent or less.

S = Soluble Amino Acids.

P = Protein bound Amino Acids.

TABLE XXXIII

RECOVERY OF RADIOACTIVITY FROM METHIONINE-2-C^{14}[a]

	Diet 1		Diet 2		Diet 3		Diet 4		Diet 5	
	S	P	S	P	S	P	S	P	S	P
Lysine	4.75	T	4.23	1.23	2.11	1.77	T	T	2.40	T
Histidine	2.36	3.68	3.52	T	3.66	1.77	3.84	T	1.37	5.17
Arginine	T	T	5.63	T	12.22	5.31	3.84	T	7.23	12.08
Aspartic Acid	8.70	9.24	4.23	8.98	26.61	21.26	5.74	44.75	6.20	3.45
Threonine	5.04	3.32	1.27	8.78	11.41	3.54	8.14	11.05	8.26	T
Serine	17.73	1.85	T	18.58	3.26	5.31	5.74	6.63	8.22	8.62
Glutamic Acid	4.75	5.82	6.23	9.81	4.87	T	15.30	4.97	T	12.93
Proline	3.69	T	4.23	4.90	8.15	T	9.09	T	T	T
Glycine	10.44	1.38	11.26	4.90	T	T	T	T	9.30	T
Alanine	6.86	1.11	3.52	12.86	T	T	T	T	4.13	18.11
Cysteine	T	T	5.07	T	T	T	T	T	26.47	T
Valine	1.66	T	4.23	9.19	T	11.50	T	14.91	9.63	2.15
Methionine	15.86	67.62	17.42	10.20	13.05	31.86	6.70	6.63	8.26	8.62
Isoleucine	6.96	2.08	7.06	3.06	3.26	4.42	9.56	2.21	T	25.86
Leucine	1.06	1.62	5.64	1.80	8.15	4.42	T	8.84	1.34	T
Tyrosine	T	T	4.92	5.71	3.25	8.86	7.65	T	1.03	2.15
Phenylalanine	3.80	T	3.94	T	T	T	13.39	T	3.43	T
B alanine	6.33	T	6.77	T	T	T	11.00	T	2.73	T

[a]Numbers represent a percent of the total radioactivity recovered from each analysis.

T = 1 percent or less.

S = Soluble Amino Acids.

P = Protein bound Amino Acids.

from methionine-2-C^{14}. Other work in this laboratory with threonine has revealed recovery of labeled carbon in lysine (Teekell et al., 1966). This would tend to support the hypothesis of limited lysine synthesis.

The recovery of labeled amino acid from methionine-2-C^{14} could be explained in approximately the same manner as was the $C^{14}H_3$ methionine. Once the label is incorporated into an intermediate of the citric acid cycle, the appearance of radioactivity in most of the amino acids can be accounted for. Also production of CO_2 by the citric acid cycle would allow one-carbon transfers to occur and thence become a component of many metabolites.

It was believed that separations of the amino acids would indicate if there was any preferential use of methionine at a particular dietary level. However, the data did not reveal any particular patterns from which definite conclusions could be drawn. At the 0.24 dietary level of methionine it was noted that the amount of labeled carbon recovered from the $C^{14}H_3$ methionine was substantially less than the percent recovery from the methionine-2-C^{14}. If the proteins of the liver were synthesized from the soluble protein fraction this then would indicate that transmethylation was occurring prior to protein synthesis. This of course assumes little if any transmethylation of methionine after its incorporation into protein. However, it is hard to justify these statements with the observation that no improvement in protein synthesis in the muscle was obtained above

0.54 percent. If the requirement for transmethylation was satisfied before that of protein synthesis, and the requirement for protein synthesis was satisfied at 0.54 percent methionine, then optimum weight gain should have occurred at this level instead of at the 0.84 percent level.

It is possible that transmethylation does occur prior to protein synthesis and that only a slightly larger amount of supplemental methionine would have resulted in maximum weight gain and feed conversion. To determine conclusively whether this situation actually exists an additional experiment would have to be undertaken utilizing various levels of methionine between 0.54 and 0.84 percent.

One point that should be definitely brought in this discussion is that very wide distribution of not only the C^{14}-H_3-methionine but also of the methionine-2-C^{14} was noted. This would indicate that rapid metabolism was occurring by way of many many pathways.

SUMMARY

It has been amply demonstrated that methionine functions as a component of protein and/or as a source of labile methyl groups for one-carbon metabolism. Therefore, this experiment was conducted to determine if a change in the dietary level of DL-methionine resulted in a preferential use of this material for one or the other of these functions.

It was found that a dietary methionine level of 0.84 percent was optimum for weight gain and feed conversion. Superoptimum dietary levels of methionine did not appear too dissimilar from the 0.84 percent level. The largest effect noted from administration of above-optimum levels was a slight decrease in weight gain.

It was noted that feed consumption was depressed by dietary methionine levels which were either deficient or excessive even though the energy content was constant. It was observed that the 0.54 percent level of methionine was adequate for protein synthesis. This was determined by recovery of labeled carbons from the muscle.

It was further noted that transmethylation was occurring at a rapid rate at all levels of methionine supplementation. This was particularly evident at the 0.24 percent level of dietary methionine. This would suggest that

transmethylation is occurring previous to protein formation or that these functions occurred simultaneously.

The liver data indicated that, as in the muscle, transmethylation was occurring at a rapid rate, especially at the two lower levels of methionine supplementation. Of particular interest was the low percent recovery of radioactivity from $C^{14}H_3$ methionine from the 0.54 percent level. At this level of supplementation the ratio between the percent recovery of radioactivity from the two isotopically labeled carbons was greater than what was observed at the 0.24 percent level.

Analysis of the kidney indicated that the 0.54 level of methionine-2-C^{14} resulted in a high recovery value of labeled carbon. In the previous discussion it was reported that these data could well indicate rapid transmethylation at the 0.24 percent level.

Blood samples were analyzed. These data suggested only that circulating blood levels of amino acids were not an accurate measure of nutritional adequacy but were merely a reflection of dietary intake.

The soluble protein fractions of the livers were separated into individual amino acids by ion exchange chromatography. Subsequently, these amino acids were analyzed for labeled carbon and it was noted that there was widespread distribution of the radioactivity which originated from methionine.

The question of possible synthesis of lysine by the

chick was suggested as a result of these analyses.

In general these data would indicate that there is no true preference in utilization of dietary methionine for one-carbon metabolism or as a component of protein. This is basically what would be expected in that both functions are essential to the maintenance of life. However, the author wishes to make some basic statements regarding the interpretation of these experiments. From what has been presented it is evident that transmethylation occurs under even the most deficient dietary regimes and it is also evident that at least limited protein synthesis occurs at these same levels but it would appear from the information derived from the various organs that the majority of a limited supply of methionine would be utilized for one-carbon metabolism and the remaining material involved in protein synthesis. This, however, should not be considered an overriding preference but merely a division of nutrients based on the organism's most critical needs. When one considers the information from this aspect many of the intricate patterns found in these experiments can be resolved and the reason for at least two possible explanations of these data can be seen.

CONCLUSIONS

The results obtained from this study indicated that the following conclusions may be warranted.

1. A diet containing 0.09 percent cysteine and 0.84 percent methionine was optimum for growth and feed conversion.

2. Feed consumption can be depressed by deficient or excessive amounts of methionine.

3. The efficiency of utilization of methionine was decreased as the amount in the diet was increased.

4. In these studies blood levels tended to reflect eating habits and not dietary levels.

5. Four hours after administration labeled carbon from methionine was widely distributed and could be found in many metabolites.

6. It appeared that rapid transmethylation was occurring at all dietary levels and in particular at the lowest level of supplementation (0.24 percent).

7. Above optimum levels of dietary methionine resulted in a growth depression. This was considered to be indicative of an imbalance in the amino acid levels.

8. Metabolism of labeled methionine indicated that a steady state equilibrium between one-carbon metabolism and

protein synthesis was reached above a dietary level of 0.84 percent.

9. A true preferential utilization of methionine at a particular dietary level was not demonstrated.

SELECTED REFERENCES

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Almquist, H. J., 1949. Amino acid balance at supernormal dietary levels. Proc. Soc. Exp. Biol. Med. 72:179-180.

_____, 1965. Nutritional values of methionine isomers and hydroxy analogue. Nutr. Conf. for Feed Mfg. Pp. 7-10.

Balloun, S. L., 1962. Lysine, arginine, and methionine balance of diets for turkeys to 24 weeks of age. Poultry Sci. 41:417-424.

Bauriedel, W. R., 1963. The effect of feeding D-methionine on the D-amino acid oxidase activity of chick tissue. Poultry Sci. 42:214-217.

Bender, A. E., and H. A. Krebs, 1950. The oxidation of various synthetic amino acids by mammalian D-amino acid oxidase, L-amino acid oxidase of cobra venom and the L- and D-amino acid oxidase of Neurospora crassa. Biochem. J. 46:210-219.

Berg, C. P., 1959. Utilization of D-amino acids in protein and amino acid nutrition. Amino Acid Advances. Academic Press, New York, New York. Pp. 57-96.

Bird, F. H., 1952. A comparison of methionine and two of its analogues in the nutrition of the chick. Poultry Sci. 31:1095-1096.

Block, R. J., and K. W. Weise, 1956. Amino Acid Handbook. Charles C. Thomas, Springfield, Ill.

Bremer, J., and D. M. Greenberg, 1960. Biosynthesis of choline in vitro. Biochem. Biophys. Acta. 37:173-175.

Carew, L. B., Jr., and F. W. Hill, 1961. Methionine deficiency and energy utilization in chicks. J. Nutrition, 74:185-187.

Christensen, H. N., 1962. Biological Transport. W. A. Benjamin, Inc., New York, New York. Pp. 114-117.

Christensen, H. N., T. R. Riggs, H. Fischer, and I. M. Palatine, 1952. Amino acid concentration by a free cell neoplasm: relations among amino acids. J. Biol. Chem. 198:1-5.

du Vigneaud, V., J. P. Chandler, A. W. Moyer, and D. M. Keppel, 1939. The effect of choline on the ability of homocystine to replace methionine in the diet. J. Biol. Chem. 131:57-76.

Featherston, W. R., and E. L. Stephenson, 1960. Dietary interrelations between methionine, glycine, choline, protein level and energy content of the chick diet. Poultry Sci. 29:1023-1029.

Fell, R. V., W. S. Wilkinson, and A. B. Watts, 1959. The utilization by the chick of D- and L-amino acids in liquid and dry diets. Poultry Sci. 38:1203-1204.

Gibson, Q. H., H. Newey, D. H. Smyth, and B. C. Whaler, 1954. Synthesis of L-alanine and L-leucine from their unnatural enantiomorphs. J. Physiol. 125:65p.

Gordon, R. S., and I. W. Sizer, 1955. The biological equivalence of methionine hydroxy analogue. Poultry Sci. 34:1198-1201.

Jervis, E. L., and D. H. Smyth, 1959. Competition between enantiomorphs of amino acids during intestinal absorption. J. Physiol. 145:57-65.

Johnson, W. A., 1966. Unpublished data. Louisiana State University.

Kamath, S. H., and C. P. Berg, 1964. Antagonism of poorly invertible D-amino acids toward growth promotion by readily invertible D-amino acids. J. Nutrition, 82:237-242.

Kamin, H., and P. Handler, 1952. Effect of presence of other amino acids upon intestional absorption of single amino acids in the rat. Am. J. Physiol. 169:305-308.

Klose, A. A., and H. J. Almquist, 1941. Methionine in the diet of the chick. J. Biol. Chem. 138:467-469.

Langer, B. W., Jr., and F. H. Kratzer, 1964. Labile methyl group biosynthesis: 1. Dietary methionine, choline, vitamin B_{12}, and their effects on growth and methyl group biosynthesis *in vitro*. Poultry Sci. 43:127-135.

Machlin, L. J., and R. S. Gordon, 1959. Equivalence of methionine hydroxy analogue and methionine for chickens fed low protein diets. Poultry Sci. 38:650-652.

_____, J. T. Jackson, A. H. Lankenau, and B. P. Pearson, 1954. Uptake of S^{35} in the feathers, gizzard lining, liver, and muscle of growing chickens after injection of radioactive methionine of sulfate. Poultry Sci. 33:234-238.

Marrett, L., H. R. Bird, and M. L. Sunde, 1964. The effect of different isomers of methionine on growth of chicks fed amino acid diets. Poultry Sci. 43:1113-1118.

Metthews, D. M., and D. H. Smyth, 1954. The intestinal absorption of amino acid enantiomorphs. J. Physiol. 128:67p.

Moore, S., D. H. Spackman, and W. H. Stein, 1958. Chromatography of amino acids on sulfonated polystyrene resins. An improved system. Anal. Chem. 30:1185-1190.

_____, and W. H. Stein, 1951. Chromatography of amino acid on sulfonated polystyrene resins. J. Biol. Chem. 192:663-681.

_____, and W. H. Stein, 1954. Procedures for chromatographic determination of amino acids on four per cent cross-linked sulfonated plystyrene resins. J. Biol. Chem. 211:893-906.

Newell, G. W., and C. S. Shaffner, 1950. Blood volume determinations in chickens. Poultry Sci. 29:78-87.

Oxender, D. L., and H. N. Christensen, 1963. Distinct mediating system for the transport of neutral amino acids by the Ehrlich cell. J. Biol. Chem. 238:3686-3698.

Qullin, E. C., G. F. Combs, R. D. Creek, and G. L. Romosier, 1961. Effect of choline on the methionine requirement of broiler chickens. Poultry Sci. 40:639-645.

Rasheed, A. A., and J. E. Oldfield, 1964. Effect of clipping wings and tails in chicks: 2. Alterations of Methionine requirement. Poultry Sci. 43:318-329.

Rosenberg, H. R., and J. T. Baldini, 1957. Effect of dietary protein level on the methionine energy relationship of broiler diets. Poultry Sci. 36:247-252.

_____, J. T. Baldini, M. L. Sunde, H. R. Bird and T. D. Runnels, 1955. The concomitant use of fat and methionine in broiler diets. Poultry Sci. 34:1308-1313.

Simmonds, S., M. Cohn, J. P. Chandler, and V. du Vigneaud, 1943. The utilization of the methyl group of choline in the biological synthesis of methionine. J. Biol. Chem. 140:519-523.

Smith, G. N., P. D. Ludwig, K. C. Wright, and W. R. Bauriedel, 1964. Pesticide residue methodology, simple apparatus for combustion of samples containing C^{14}-labeled pesticides for residue analysis. J. Agri. and Food Chem. 12:172-175.

Spackman, D. H., W. H. Stein, and S. Moore, 1958. Automatic recording apparatus for use in the chromatography of amino acid. Anal. Chem. 30:1190-1206.

Steel, R. G. D., and J. H. Torrie, 1960. Principles and Procedures of Statistics. New York: McGraw-Hill Book Company, Inc.

Sullivan, T. W., and H. R. Bird, 1957. Effect of quantity and source of dietary nitrogen of the utilization of the hydroxy analoque of methionine and glycine by chicks. J. Nutrition, 62:143-150.

Teekell, R. A., E. N. Knox, and A. B. Watts, 1966. Absorption and deposition of threonine-C^{14} in chicks. Poultry Sci. 45:1132.

Tipton, H. C., B. C. Dilworth, and E. J. Day, 1965. The relative biological value of DL-methionine and methionine hydroxy analogue in chick diets. Poultry Sci. 44:987-992.

Waibel, P. E., 1959. Methionine and lysine in rations for turkey poults under various dietary conditions. Poultry Sci. 38:712-720.

White, A., and E. F. Beach, 1937. The role of cystine, methionine, and homocystine in the nutrition of the rat. J. Biol. Chem. 122:219-226.

Williams, J. N., Jr., 1964. Response of the liver to prolonged protein depletion. IV Protection of succinic oxidase and succinic dehydrogenase by dietary methionine and cystine in a protein free diet. J. Nutrition, 82:57-60.

Wiseman, G., 1955. Preferential transference of amino acids from amino acid mixtures by sacs of everted small intestine of the golden hamster (Mesocricetus auratus). J. Physiol. 127:414-422.

Wretland, K. A. J., 1952. The effect of D-amino acid on the steronaturalization of D-methionine. Acta Physiol. Scand. 25:267-275.

AUTOBIOGRAPHY

I was born May 3, 1940, in Monticello, Arkansas, the son of Walton and Elaine Cardin. I attended elementary and high school in Beebe, Arkansas, and was graduated in May, 1958.

I entered the University of Arkansas in September, 1958, and graduated with a Bachelor of Science degree in Animal Nutrition in January, 1963. In January, 1964, I received a Master of Science degree in Poultry Nutrition from the same institution.

In September, 1963, I was admitted to the Graduate School of Louisiana State University and received a graduate assistantship in the Poultry Science Department. Since that time I have pursued a curriculum leading toward a Doctor of Philosophy degree with a major in Poultry Nutrition and a minor in Biochemistry.

I am married to the former Mary Nell Freeman of Pine Bluff, Arkansas, and we have a daughter, Tracy, who was born November 29, 1964.

EXAMINATION AND THESIS REPORT

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Major Field: Poultry Nutrition

Title of Thesis: Metabolism of Methionine by Certain Tissues of the Intact Chick

Approved:

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